

# Effect of $\text{CaCl}_2$ hydrothermal treatment on the bone bond strength and osteoconductivity of Ti–0.5Pt and Ti–6Al–4V–0.5Pt alloy implants

Masaharu Nakagawa · Jyunichi Yamazoe

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**Abstract** To achieve osteoconductivity, Ti–0.5Pt and Ti–6Al–4V–0.5Pt alloys were hydrothermally treated at 200°C in 10 mmol/l  $\text{CaCl}_2$  aqueous solution for 24 h (HT-treatment). We conducted histological investigations of the HT-treated materials by using Wistar strain rats (SD rats) to evaluate the usefulness of the treatment. To measure the bone bond strength, the specimens were implanted in the tibia of SD rats, and a pull-out test was conducted. From the early postoperative stages, direct bone contact was obtained for the HT-treated implants. Within 1–4 weeks of implantation, the bone contact ratios and bone bond strengths of the HT-treated implants were higher than those of the non-treated implants. The Ti–0.5Pt and Ti–6Al–4V–0.5Pt alloys with HT-treatment showed the potential to develop a new implant with a high bone bond strength and rapid osteoconduction.

## 1 Introduction

Titanium (Ti) has been used clinically in dental implants and in orthodontic implant anchors because of its superior corrosion resistance and biocompatibility. When substantial strength is required, a Ti–6Al–4V alloy, which has higher mechanical strength than Ti, is used as the implant material. In general, because Ti is non-toxic and does not cause any adverse tissue reactions, it is considered to be a bioinert

material. However, the direct bonding of Ti and bone is not always satisfactory [1]. Histological studies revealed that direct bonding between Ti and bone was not readily achieved, and intervening fibrous tissue was observed [2, 3]. In addition, adequate osseointegration may not be achieved until healing has progressed over a period of months, and during this period, micro-movements will be caused by premature loading because of the existence of fibrous tissue between the bone and the implant [4, 5]. In order to improve the bioactivity of Ti to achieve better osteoconduction or faster bone formation, various surface modifications have been developed. When bioactive materials are implanted into bone, a so-called bone-like apatite layer is formed on the materials' surfaces, and these materials form a chemical bond with the bone through this layer [6–8]. Treatment of Ti with alkali and heat ( $\text{NaOH} + \text{heat}$ ) is of note because it changes the properties of Ti, converting it from a bioinert to a bioactive state [9–12]. We investigated the use of hydrothermal treatment of Ti in  $\text{CaCl}_2$  aqueous solution for achieving faster bone formation. When Ti was hydrothermally treated at 200°C in the presence of 10 mmol/l  $\text{CaCl}_2$  aqueous solution for 24 h, we found that  $\text{Ca}^{2+}$  bonded to the Ti surface, and the thickness of the titanium oxide layer increased [13]. Thus, the Ti surface has a  $\text{Ca}^{2+}$ -bonded titanium oxide layer. This  $\text{CaCl}_2$ -hydrothermally treated Ti ( $\text{CaCl}_2$ -HT-Ti) elicited bone-like apatite formation as early as 36 h after immersion in simulated body fluid. In a cell-based experiment using osteoblast-like cells (MC3T3-E1), we found that the  $\text{CaCl}_2$ -HT-Ti surface promoted the adhesion and proliferation of MC3T3-E1 cells [14].

The corrosion resistance of Ti is significantly reduced in fluoride-containing environments since such environments destroy the passive surface film of Ti [15–20]. Ti and the Ti–6Al–4V alloy tend to corrode in the presence of a small amount of fluoride (less than 0.1% NaF) under acidic

M. Nakagawa (✉) · J. Yamazoe  
Department of Biomaterials, Faculty of Dental Science, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan  
e-mail: nakagawa@dent.kyushu-u.ac.jp

conditions [21–24]. Acidulated phosphate fluoride (APF) solutions and fluoride-containing toothpastes and mouth rinses are often used to prevent dental caries. The fluoride concentration in these substances is between 250 and 10,000 ppm, and their pH ranges from  $\sim 3.5$ –7.0 [15]. If such toothpastes and mouth rinses are continuously used, Ti implants or orthodontic Ti implant anchors might corrode. Our previous studies showed that the addition of a small amount of Pt or Pd to Ti is effective in improving corrosion resistance [22]. In particular, Ti–0.5 wt% Pt alloys (Ti–0.5Pt) have an optimal alloy composition that improves fluoride corrosion resistance in a fluoride-containing environment [21, 22]. Moreover, we found that Ti–6Al–4V–0.5 wt% Pt alloys (Ti–6Al–4V–0.5Pt) have high corrosion resistance and high mechanical strength [25].

In this study, we hydrothermally treated Ti–0.5Pt and Ti–6Al–4V–0.5Pt alloys at 200°C in 10 mmol/l CaCl<sub>2</sub> aqueous solution for 24 h, and we histologically investigated the resultant materials in Wistar strain rats to evaluate the usefulness of the treatment. To measure the bone bond strength, we conducted pull-out tests. We examined the possibility of developing an oral implant material with high bone bond strength and rapid osteoconduction.

## 2 Materials and methods

### 2.1 Sample preparation

In this study, we examined commercially pure titanium (Nilaco Corporation, Tokyo, Japan); Ti–6Al–4V (Daido Steel Co. Ltd., Nagoya, Japan), which is presently used in clinical practice; and the experimental alloys Ti–0.5 wt% Pt (Ti–0.5Pt) and Ti–6Al–4V–0.5 wt% Pt (Ti–6Al–4V–0.5Pt). Specimens of Ti and Ti–6Al–4V were prepared from the as-received materials by using an argon-arc casting machine (Cyclarc II, J. Morita Co., Kyoto, Japan). The alloy specimens were melted using an argon arc and were maintained in a molten condition for 60 s before they were cast into the mold (Titavest CB, J. Morita Co.). By using the Cyclarc II argon-arc casting machine, the Ti–0.5Pt and Ti–6Al–4V–0.5Pt alloys were prepared from pure Ti and the Ti–6Al–4V alloy, respectively, and 99.95% Pt. The specimens were melted twice by exchanging the top and bottom materials in the crucible, and they were then cast into the mold (Titavest CB). After casting, the surface of the specimens was polished using a waterproof grinding paper (#1500), and the specimens were washed in ethanol by using an ultrasonic washing machine. These cylindrical specimens of diameter 1.0 mm and length 3.5 mm were used for histological examination. They were divided into two groups each: (1) non-treated Ti and the Ti–0.5Pt, Ti–6Al–4V, and

Ti–6Al–4V–0.5Pt alloys; (2) Ti and the Ti–0.5Pt, Ti–6Al–4V, and Ti–6Al–4V–0.5Pt alloys treated with 10 mmol/l CaCl<sub>2</sub> aqueous solution at 200°C for 24 h (HT-treatment). All the specimens were washed using distilled water and were sterilized with 70% ethanol by using an ultrasonic washing machine for 15 min.

### 2.2 Surgical procedure

All the animal experiments were performed according to the Kyushu University guidelines for animal experiments. Young (8 weeks old) male Wistar rats (SD rats) that were fed water and laboratory diet ad libitum were used. The rats were anesthetized using intraperitoneal ketamine (Sankyo Co. Ltd., Tokyo, Japan) and xylazine (Bayer Health-Care Co. Ltd. Tokyo, Japan) injection. After shaving and cleaning, the tibia was exposed, and a hole (1.0 mm in diameter) was drilled using a dental bur (#1) and dental reamers (#90–100) under saline irrigation for cooling and cleaning. The hole was 10 mm below the knee joint. With slight pressure, the cylindrical specimens were placed in the holes, and the surgical wound was closed with 4–0 silk sutures.

### 2.3 Histological examination

Decalcified semi-thin sections (1  $\mu$ m) were histologically examined. On postoperative days 7, 14, and 28 ( $N = 5$  for each group and for each time point), the rats were sacrificed under systemic anesthesia by using an overdose of pentobarbital sodium (Schering-Plough Co. Ltd., New Jersey, USA). They were then perfused with heparinized normal saline for 3 min and fixed with 100 ml of 4% paraformaldehyde solution. The dissected tibia implant specimens were fixed for three extra days. The specimens were subsequently decalcified in a solution containing 5% EDTA and 4% saccharose for approximately 1 month. The tibia was then carefully cut using a razor blade along the long axis of the Ti cylinder [26]. Finally, all the specimens were dehydrated in serial concentrations of ethanol (50–100%) and QY-1 (*n*-butyl glycidyl ether) and then embedded in epoxy resin (Quetol 651, Nisshin EM Co. Ltd., Tokyo, Japan). The specimens were cut into 1- $\mu$ m-thick sections by using an ultramicrotome (Reichert Ultracut S, Hitachi High-Tech-nologies Corporation, Tokyo, Japan). These sections were stained with toluidine blue for observation under a light microscope.

### 2.4 Histomorphometrical evaluation

The length of direct bone contact was measured on the histological photograph, and the bone contact ratio was

calculated using the following formula: bone contact ratio = length of direct bone contact/total length of the implant [27]. The results were statistically analyzed using ANOVA and Scheffe's test at a significance level of 1%.

## 2.5 Bone bond strength evaluation

The bond strength between the bone and the specimen was measured by a pull-out test. The maximum pull-out strengths were determined using a universal testing machine (Autograph AGS-J, Shimadzu Co., Kyoto, Japan). The dissected tibial implant specimens were fixed in 4% paraformaldehyde solution for 12 h. As shown in Fig. 1, the specimen-implanted bone was held in a vise and the protruding specimen was gripped using a chuck (pantograph-type chuck, Shimadzu Co.). The specimen was vertically pulled from the bone at a crosshead speed of 1.0 mm/min. The bond strengths were calculated using the following formula: maximum pull-out strength/interface area of the implanted specimen, including the intramedullary part. Five specimens under each condition were tested. The results were statistically analyzed using ANOVA and Scheffe's test at a significance level of 5%.



**Fig. 1** The pull-out test device (pantograph-type chuck, Shimadzu, Kyoto, Japan)

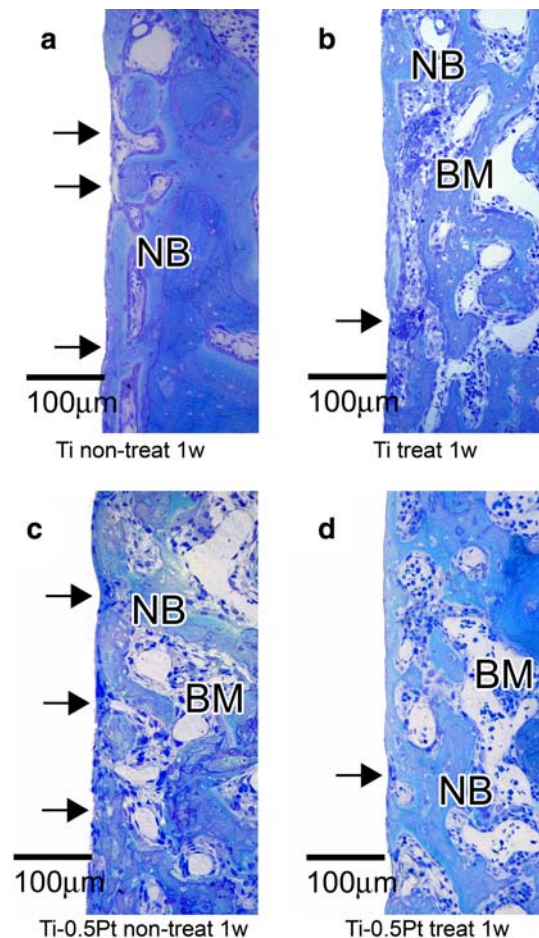
## 3 Results

### 3.1 Histological observations

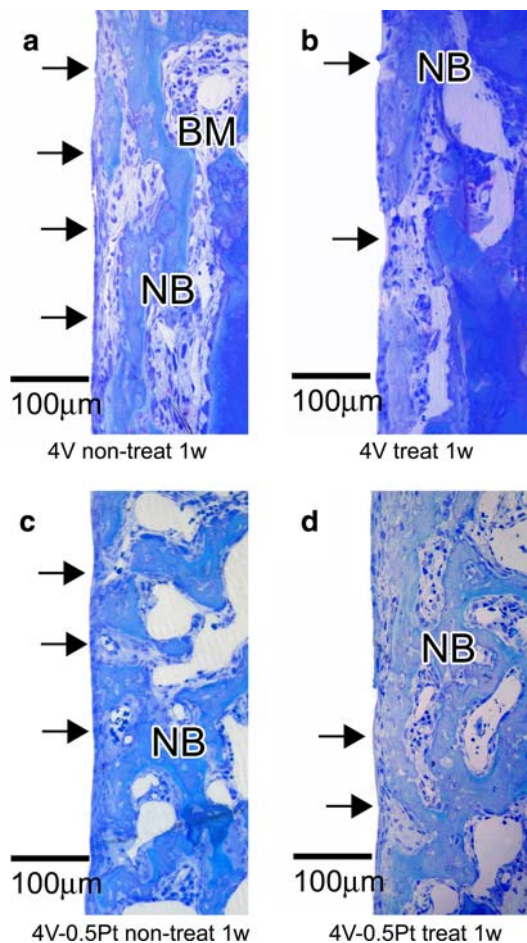
Inflammatory reactions were not observed in any of the groups during the entire implantation period.

#### 3.1.1 One week after implantation

New bone was formed around the Ti, Ti-0.5Pt, Ti-6Al-4V, and Ti-6Al-4V-0.5Pt implants in the cortical and in the intramedullary part of the implants, and woven bone was observed (Figs. 2, 3). Thin fibrous tissues were present at the implant-bone interface of the non-treated specimens (indicated by an arrow). With regard to the non-treated specimens, direct bone contact was found only in a few areas (Figs. 2a, c and 3a, c). On the other hand, the implant-bone interface of the HT-treated Ti and Ti-0.5Pt implants exhibited direct bone contact over large areas



**Fig. 2** Micrographs taken at 1 week after implantation. **a** Non-treated Ti, **b** Ti treated hydrothermally in 10 mmol/l  $\text{CaCl}_2$  solution at 200°C for 24 h, **c** non-treated Ti-0.5Pt, and **d** Ti-0.5Pt treated hydrothermally in 10 mmol/l  $\text{CaCl}_2$  solution at 200°C for 24 h. Ti: implant, NB: new bone, BM: bone marrow, arrow: fibrous tissue

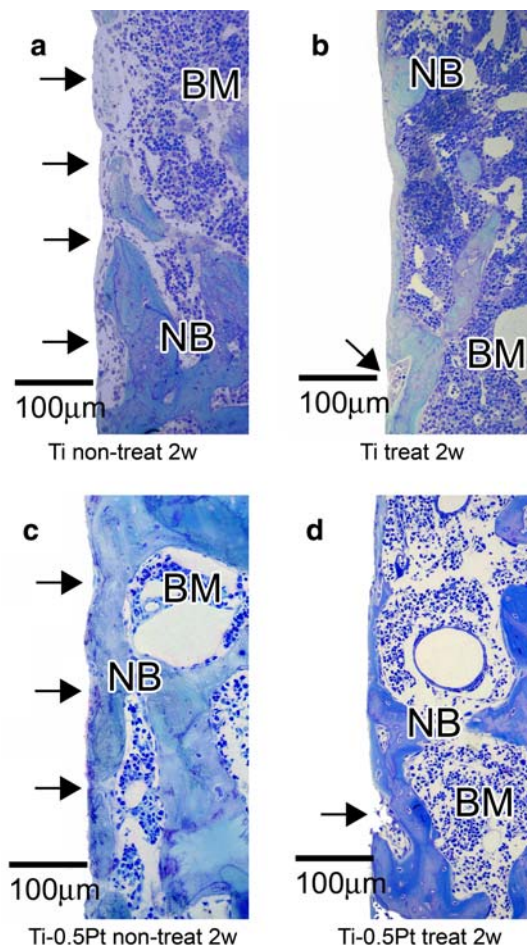


**Fig. 3** Micrographs taken at 1 week after implantation. **a** Non-treated Ti-6Al-4V, **b** Ti-6Al-4V treated hydrothermally in 10 mmol/l CaCl<sub>2</sub> solution at 200°C for 24 h, **c** non-treated Ti-6Al-4V-0.5Pt, and **d** Ti-6Al-4V-0.5Pt treated hydrothermally in 10 mmol/l CaCl<sub>2</sub> solution at 200°C for 24 h. Ti: implant, NB: new bone, BM: bone marrow, arrow: fibrous tissue

(Fig. 2b, d). The areas of direct bone contact for the HT-treated Ti-6Al-4V and Ti-6Al-4V-0.5Pt implants were smaller than those of the HT-treated Ti and Ti-0.5 implants (Fig. 3b, d). Implants of the Ti alloys with and without Pt showed a similar tendency (Figs. 2, 3).

### 3.1.2 Two weeks after implantation

The woven bone around the HT-treated specimens changed into lamellar bone that uniformly covered most parts of the implant surface (Figs. 4b, d and 5b, d). In the case of non-treated specimens, although the areas of direct bone contact increased slightly, the intervening fibrous tissue layer remained at the bone-titanium interface (indicated by an arrow), and this layer thickened compared with that observed at 1 week in vivo. Two weeks after implantation, the areas of direct bone contact for the HT-treated Ti-6Al-4V and Ti-6Al-4V-0.5Pt implants increased as remarkably



**Fig. 4** Micrographs taken at 2 weeks after implantation. **a** Non-treated Ti, **b** Ti treated hydrothermally in 10 mmol/l CaCl<sub>2</sub> solution at 200°C for 24 h, **c** non-treated Ti-0.5Pt, and **d** Ti-0.5Pt treated hydrothermally in 10 mmol/l CaCl<sub>2</sub> solution at 200°C for 24 h. Ti: implant, NB: new bone, BM: bone marrow, arrow: fibrous tissue

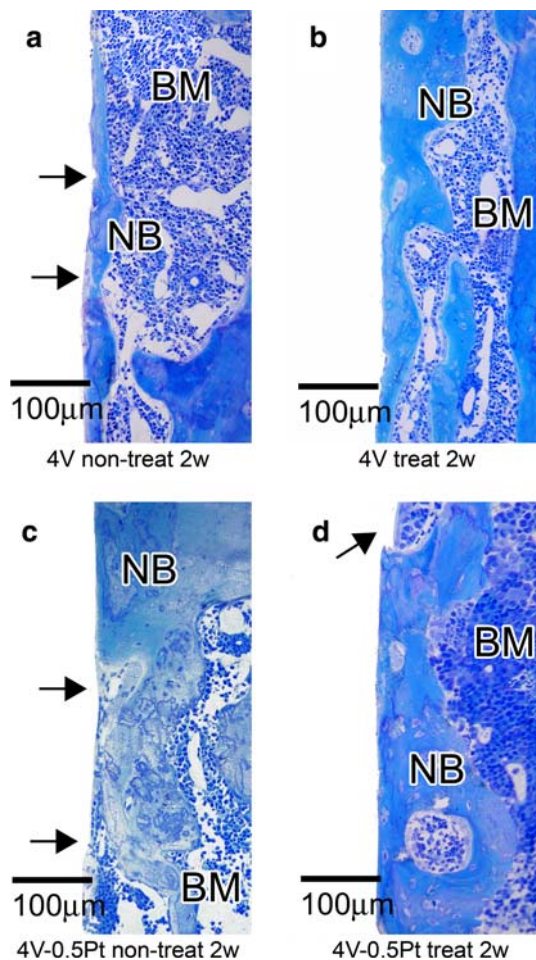
as those for HT-treated Ti and the Ti-0.5Pt implants (Fig. 5b, d).

### 3.1.3 Four weeks after implantation

The surfaces of the HT-treated specimens were almost entirely covered with lamellar bone (Figs. 6b, d and 7b, d). The areas of direct bone contact also increased considerably in the non-treated specimens, but fibrous tissue was still observed at the bone-implant interface (Figs. 6a, c, and 7a, c, indicated by an arrow).

## 3.2 Histomorphometrical evaluation

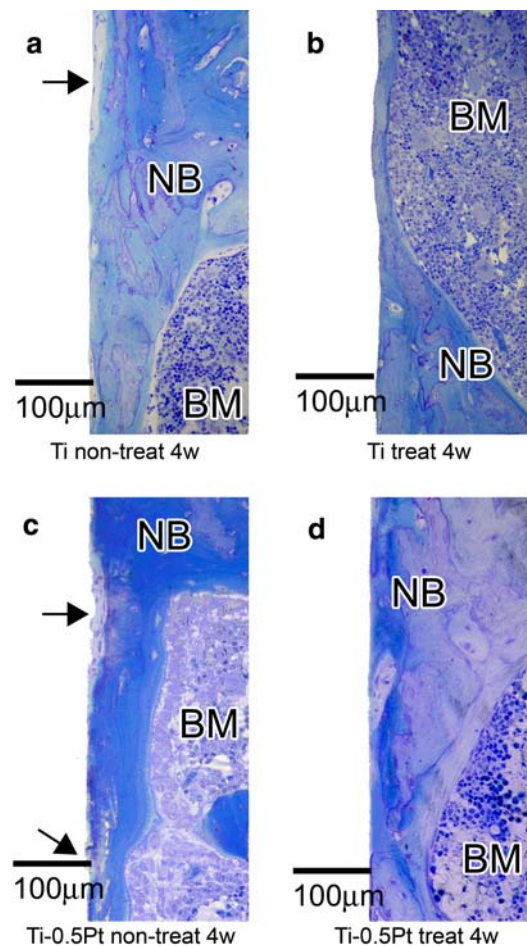
Figure 8 shows the direct bone contact ratio (area of direct bone contact interfaces/area of all interfaces) at the bone-implant interface. One week after implantation, the bone contact ratios of the HT-treated Ti and Ti-0.5Pt implants were substantially higher (Ti = 55.2% ± 11.4%,



**Fig. 5** Micrographs taken at 2 weeks after implantation. **a** Non-treated Ti-6Al-4V, **b** Ti-6Al-4V treated hydrothermally in 10 mmol/l CaCl<sub>2</sub> solution at 200°C for 24 h, **c** non-treated Ti-6Al-4V-0.5Pt, and **d** Ti-6Al-4V-0.5Pt treated hydrothermally in 10 mmol/l CaCl<sub>2</sub> solution at 200°C for 24 h. Ti: implant, NB: new bone, BM: bone marrow, arrow: fibrous tissue

Ti-0.5Pt = 43.2% ± 10.0%) than those of the non-treated Ti and Ti-0.5Pt implants (Ti = 5.7% ± 3.4%, Ti-0.5Pt = 7.8% ± 2.2%). The bone contact ratios of the non-treated Ti-6Al-4V and Ti-6Al-4V-0.5Pt implants were very low (almost 0), while those of the HT-treated Ti-6Al-4V and Ti-6Al-4V-0.5Pt implants were only 3.8% ± 2.6% and 8.0% ± 2.1%, respectively.

During the implantation period, up to 4 weeks, the bone contact ratio of the non-treated Ti, Ti-0.5Pt, Ti-6Al-4V, and Ti-6Al-4V-0.5Pt implants increased to 57.4% ± 9.9%, 58.1% ± 3.2%, 45.3% ± 3.9%, and 48.9% ± 6.0% ( $P < 0.01$ ), respectively. On the other hand, in the implantation period, up to 2 weeks, the bone contact ratios of the HT-treated Ti, Ti-0.5Pt, Ti-6Al-4V, and Ti-6Al-4V-0.5Pt implants increased dramatically to 88.1% ± 8.9%, 89.1% ± 3.1%, 66.4% ± 2.5%, and 75.2% ± 7.6% ( $P < 0.01$ ), respectively. Four weeks after implantation, the bone contact ratio of the HT-treated Ti, Ti-0.5Pt, Ti-6Al-4V, and



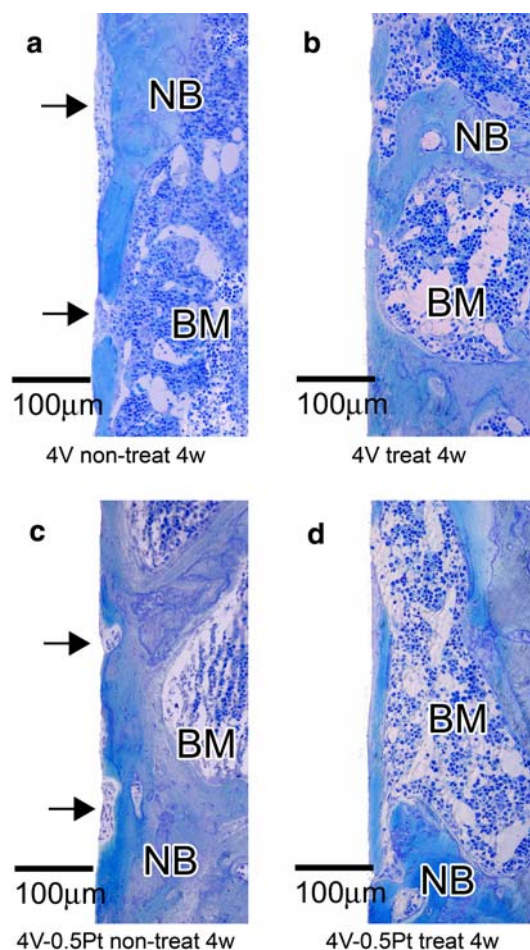
**Fig. 6** Micrographs taken four weeks after implantation. **a** Non-treated Ti, **b** Ti treated hydrothermally in 10 mmol/l CaCl<sub>2</sub> solution at 200°C for 24 h, **c** non-treated Ti-0.5Pt, and **d** Ti-0.5Pt treated hydrothermally in 10 mmol/l CaCl<sub>2</sub> solution at 200°C for 24 h. Ti: implant, NB: new bone, BM: bone marrow, arrow: fibrous tissue

Ti-6Al-4V-0.5Pt implants increased to 96.1% ± 4.8%, 92.9% ± 2.8%, 70.0% ± 4.2%, and 81.0% ± 8.1% ( $P < 0.01$ ), respectively. The addition of 0.5 wt% Pt had little influence on the bone contact ratios of the implants.

### 3.3 Pull-out test

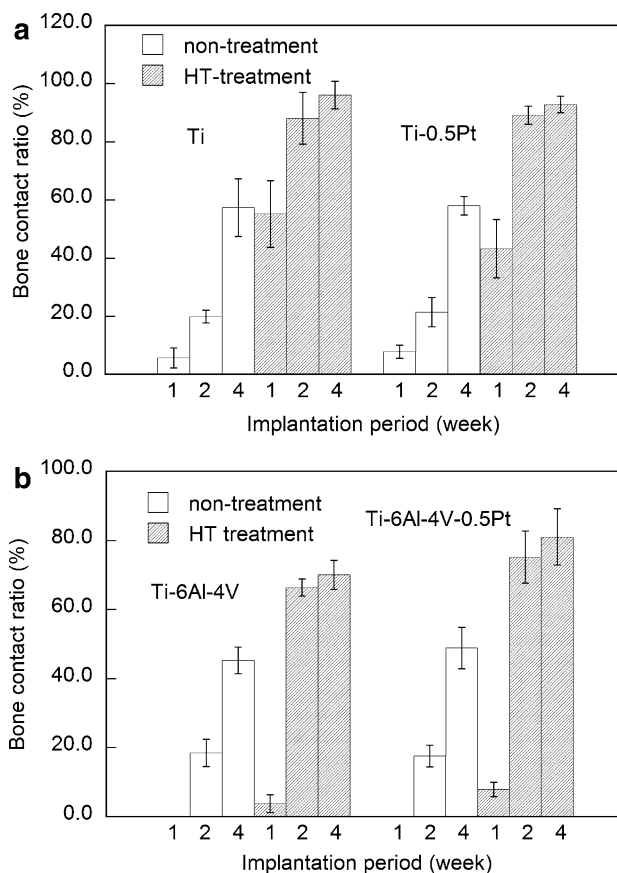
In the pull-out test, the bond strengths of the bone-implant interfaces were obtained from the peak values of the strength. Figure 9 demonstrates the mean bond strength that was obtained for each specimen after implantation for 1, 2, and 4 weeks. In all cases, the bond strength showed a tendency similar to that observed for the bone contact ratio, and it increased with the implantation period.

One week after implantation, the bond strengths of all specimens except the HT-treated Ti implant were low, ranging from 0.09 ± 0.06 to 0.34 ± 0.07 N/mm<sup>2</sup> ( $P < 0.05$ ). The bond strength of the HT-treated Ti implant



**Fig. 7** Micrographs taken at 4 weeks after implantation. **a** Non-treated Ti-6Al-4V, **b** Ti-6Al-4V treated hydrothermally in 10 mmol/l CaCl<sub>2</sub> solution at 200°C for 24 h, **c** non-treated Ti-6Al-4V-0.5Pt, and **d** Ti-6Al-4V-0.5Pt treated hydrothermally in 10 mmol/l CaCl<sub>2</sub> solution at 200°C for 24 h. Ti: implant, NB: new bone, BM: bone marrow, arrow: fibrous tissue

1 week after implantation was high, i.e.,  $0.95 \pm 0.25$  N/mm<sup>2</sup> ( $P < 0.05$ ). The bond strength was almost equal to those of the non-treated specimens 4 weeks after implantation. In the implantation period, up to 4 weeks, the bond strengths of the HT-treated specimens increased and ranged from  $1.37 \pm 0.28$  to  $1.67 \pm 0.2$  N/mm<sup>2</sup> ( $P < 0.05$ ). Those of the non-treated specimens also increased but ranged only from  $0.51 \pm 0.09$  to  $1.14 \pm 0.41$  N/mm<sup>2</sup> ( $P < 0.05$ ). The bond strength of the HT-treated Ti-0.5Pt implant was  $0.26 \pm 0.08$  N/mm<sup>2</sup> ( $P < 0.05$ ), and in the implantation period up to 1 week, it did not increase considerably. However, 2 weeks after implantation, the bond strength increased dramatically to  $1.51 \pm 0.24$  N/mm<sup>2</sup> ( $P < 0.05$ ). The bond strengths of the Ti-6Al-4V and Ti-6Al-4V-0.5Pt implants were less than those of the Ti and Ti-0.5Pt implants. Similar to the Ti-0.5Pt implant, the Ti-6Al-4V-0.5Pt implant exhibited low bond strength of  $0.14 \pm 0.1$  N/mm<sup>2</sup> ( $P < 0.05$ ) 1 week after implantation, but 2 weeks



**Fig. 8** Bone contact ratios of non-treated and HT-treated. **a** Ti, Ti-0.5Pt, **b** Ti-6Al-4V, and Ti-6Al-4V-0.5Pt implants at 1, 2, and 4 weeks after implantation

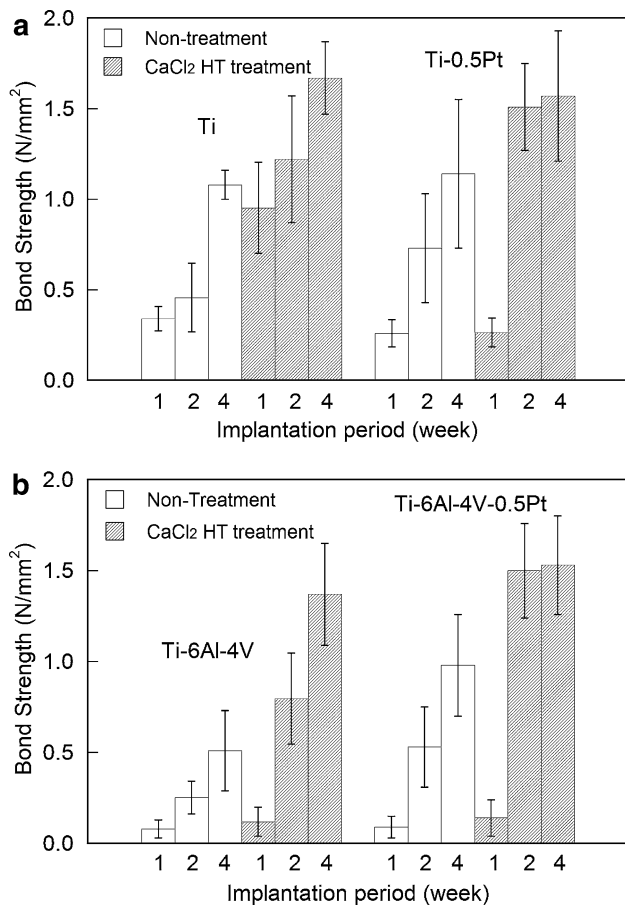
after implantation, this value increased dramatically to  $1.5 \pm 0.26$  N/mm<sup>2</sup> ( $P < 0.05$ ).

#### 4 Discussion

Our study results clearly demonstrated that not only Ti and the Ti-6Al-4V alloy but also the Ti-0.5Pt and Ti-6Al-4V-0.5Pt alloys, which acquired high corrosion resistance after hydrothermal treatment with a CaCl<sub>2</sub> aqueous solution, are superior osteoconductive materials than non-treated Ti and Ti alloys.

Mano et al. reported that the bone that formed on an apatite-coated Ti implant exposed to the intramedullary cavity was thinner than that formed on a pure Ti implant [28]. In our study, thinner bone formations were found on the surface of the HT-treated Ti and Ti implants in the intramedullary area than on the surface of non-treated Ti and Ti implants (Figs. 4b–7d). This shows that the HT-treated Ti and Ti alloys are osteoconductive.

It is known that osteoconductive materials require a negative surface charge in order to bind to Ca<sup>2+</sup> [29, 30].



**Fig. 9** Bond strengths of implant-bone interface of non-treated and HT-treated. **a** Ti, Ti-0.5Pt, **b** Ti-6Al-4V, and Ti-6Al-4V-0.5Pt implants at 1, 2 and 4 weeks after implantation

NaOH + heat-treated Ti has a negative surface charge; therefore, positive ions, including Ca<sup>2+</sup>, are attracted to its surface. Negative PO<sub>4</sub><sup>3-</sup> ions then bind to the implant surface via the attached Ca<sup>2+</sup> ions. Consequently, amorphous calcium phosphate is formed due to the reaction between the Ca<sup>2+</sup> and PO<sub>4</sub><sup>3-</sup> ions. This amorphous calcium phosphate changes to the so-called bone-like apatite, which is considered to be a requirement for osteoconductivity. If bone-like apatite is formed in this sequence, a Ti implant that has Ca<sup>2+</sup> ions on its surface would exhibit faster in vivo bone formation. We reported that bone-like apatite formation on the surface of Ti implants hydrothermally treated with the CaCl<sub>2</sub> aqueous solution was faster than those treated with NaOH + heat [13]. After immersion in the simulated body fluid for 48 h, a small amount of bone-like apatite was deposited on the surface of the Ti treated with NaOH + heat, but the deposit covered the whole surface of Ti hydrothermally treated with 10 mmol/l CaCl<sub>2</sub> aqueous solution. In this study, the bone contact ratios of the HT-treated implants were always higher than those of the non-treated implants. In particular, only

1 week after implantation, the ratios of the HT-treated Ti and Ti-0.5Pt implants were high, i.e., 55.2% ± 11.4% and 43.2% ± 10.0%, respectively, whereas those of the non-treated Ti and Ti-0.5Pt implants were 5.7% ± 3.4% and 7.8% ± 2.2%, respectively (Fig. 8). More than 2 weeks after implantation, the bone contact ratios of the HT-treated Ti and Ti implants, including the Ti-6Al-4V and Ti-6Al-4V-0.5Pt implants, were significantly (*P* < 0.01) higher than those of the non-treated Ti and Ti implants. It should be noted that the bone contact ratio of the HT-treated Ti implant was comparable to that of the apatite-coated Ti implant (blast coating method: 70% at 1 week [29], plasma spray method: 65% at 6 weeks [31], high viscosity flame spray method: 40% at 2 weeks [32]).

The bond strength of the implant-bone interface showed a tendency similar to that observed for the direct bone contact ratios. The bond strength of the non-treated specimens increased with the implantation period. In any given implantation period, the bond strengths of the HT-treated specimens were higher than those of the non-treated specimens. The bond strength of the HT-treated Ti implant 1 week after implantation (0.95 ± 0.25 N/mm<sup>2</sup>) was similar to that of the non-treated Ti implant 4 weeks after implantation (1.08 ± 0.08 N/mm<sup>2</sup>). One week after implantation, the bond strengths of the HT-treated Ti-6Al-4V (0.12 ± 0.08 N/mm<sup>2</sup>), Ti-0.5Pt (0.26 ± 0.08 N/mm<sup>2</sup>), and Ti-6Al-4V-0.5Pt (0.14 ± 0.1 N/mm<sup>2</sup>) implants were not very high, but increased dramatically (Ti-6Al-4V = 1.37 ± 0.28 N/mm<sup>2</sup>, Ti-0.5Pt = 1.57 ± 0.36 N/mm<sup>2</sup>, Ti-6Al-4V-0.5Pt = 1.53 ± 0.27 N/mm<sup>2</sup>) 4 weeks after implantation. By using the push-out test, Nishiguchi et al. reported that the bond strengths of NaOH + heat-treated Ti and Ti-6Al-4V implants 8 weeks after implantation were approximately 3 MPa (N/mm<sup>2</sup>) and 2.7 MPa, respectively [33]. Because the test methods are different, comparison between the study and ours would be inappropriate, but it is obvious that the HT-treatment used in our study sufficiently increases bone bond strength. Rapid bone contact and higher bond strength are very important for faster healing after implantation operations. In the early postoperative stages, certain implant-related problems occur often. If adequate osseointegration is not achieved, especially in the early postoperative stages, micro-movement or premature loading sometimes causes implantation failure. However, if strong bone bond formation is achieved in the early postoperative stages by using an HT-treated implant then such failures can be prevented.

The bone contact ratio and bond strength of the cytotoxic Ti-6Al-4V implant are inferior than those of the Ti implant [33]. The Ti-6Al-4V alloy is an implant material with higher mechanical strength and is useful when the implant site is anatomically restricted because the implant diameter can be reduced. The utility of the HT-treated

Ti–6Al–4V alloy might increase because it possesses osteoconductivity and high mechanical strength.

When the neck part of an implant corrodes, it breaks due to a reduction in mechanical strength, and the adhesion of plaque (biofilm) is promoted. This implant-related problem can be prevented by adding a small amount of Pt to Ti or the Ti–6Al–4V alloy, which remarkably improves corrosion resistance [21–25]. The addition of 0.5 wt% Pt did not considerably affect the osteoconductivity of the HT-treated alloy. Therefore, the HT-treated Ti–0.5Pt alloy was considered to be an osteoconductive implant material with high corrosion resistance. In our previous study, the tensile strength of the Ti–0.5Pt and Ti–6Al–4V–0.5Pt alloys were higher than those of Ti and the Ti–6Al–4V alloy [25]. Similarly, the HT-treated Ti–0.5Pt and Ti–6Al–4V–0.5Pt alloys were considered to be osteoconductive implant materials with high corrosion resistance and high mechanical strength.

Thus, it might be possible to develop new implants that have high bone bond strength and rapid osteoconduction by HT-treatment of the Ti–0.5Pt and Ti–6Al–4V–0.5Pt alloys.

## 5 Conclusions

In this study, we hydrothermally treated the Ti–0.5Pt and Ti–6Al–4V–0.5Pt alloys at 200°C in 10 mmol/l CaCl<sub>2</sub> for 24 h (HT-treatment), and we histologically investigated the resultant materials by using SD rats to evaluate the usefulness of the treatment. In order to measure the bone bond strength, we conducted a pull-out test. The following conclusions were obtained.

1. From the early postoperative stages, direct bone contact was obtained in the HT-treated implants.
2. The bone contact ratios of the HT-treated implants were always higher than those of the non-treated implants.
3. The bone bond strengths of the HT-treated implants were always higher than those of the non-treated implants.
4. It might be possible to develop new implants that have high bone bond strength and fast osteoconduction by HT-treatment of the Ti–0.5Pt and Ti–6Al–4V–0.5Pt alloys.

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## References

1. LeGeros RZ, Craig RG. Strategies to affect bone remodeling: osteointegration. *J Bone Min Res.* 1993;8:583–96.
2. Daculsi G, LeGeros RZ, Deudon C. Scanning and transmission electron microscopy, and electron probe analysis of the interface between implants and host bone. *Scan Micr.* 1990;4:309–14.
3. Hirai H, Okumura A, Goto M, Katsuki T. Histologic study of the bone adjacent to titanium bone screws used for mandibular fracture treatment. *J Oral Maxillofac Surg.* 2001;59:531–7. doi: [10.1053/joms.2001.22686](https://doi.org/10.1053/joms.2001.22686).
4. Lum LB, Beirne OR, Curtis DA. Histological evaluation of HA-coated vs. uncoated titanium blade implants in delayed and immediately loaded applications. *Int J Oral Maxillofac Implants.* 1991;6:456–62.
5. Szmukler-Moncler S, Salama H, Reingewirta Y, Dubruille JH. Timing of loading and effect of micromotion on bone-dental implant interface: review of experimental literature. *J Biomed Mater Res.* 1998;43:192–203. doi: [10.1002/\(SICI\)1097-4636\(199822\)43:2<192::AID-JBM14>3.0.CO;2-K](https://doi.org/10.1002/(SICI)1097-4636(199822)43:2<192::AID-JBM14>3.0.CO;2-K).
6. Hench LL. Bioceramics: from concept to clinic. *J Am Ceram Soc.* 1991;74:1487–510. doi: [10.1111/j.1151-2916.1991.tb07132.x](https://doi.org/10.1111/j.1151-2916.1991.tb07132.x).
7. Kokubo T. A/W glass-ceramic: processing and properties. In: Hench LL, Wilson J, editors. *An introduction to bioceramics*. Singapore: World Scientific; 1993. p. 75–88.
8. Neo M, Nakamura T, Yamamuro T, Ohtsuki C, Kokubo T. Apatite formation on three kinds of bioactive material at an early stage in vivo: a comparative study by transmission electron microscopy. *J Biomed Mater Res.* 1993;27:999–1066. doi: [10.1002/jbm.820270805](https://doi.org/10.1002/jbm.820270805).
9. Kokubo T, Miyaji F, Kim HM, Nakamura T. Spontaneous apatite formation on chemically surface treated Ti. *J Am Ceram Soc.* 1996;79:1127–9. doi: [10.1111/j.1151-2916.1996.tb08561.x](https://doi.org/10.1111/j.1151-2916.1996.tb08561.x).
10. Kim HM, Miyaji F, Kokubo T. Effect of heat treatment on apatite-forming ability of Ti metal induced by alkali treatment. *J Mater Sci: Mater Med.* 1997;8:341–7. doi: [10.1023/A:1018524731409](https://doi.org/10.1023/A:1018524731409).
11. Nishiguchi S, Nakamura T, Kobayashi M, Kim HM, Miyaji F, Kokubo T. The effect of heat treatment on bone-bonding ability of alkali-treated titanium. *Biomaterials.* 1990;20:491–500. doi: [10.1016/S0142-9612\(98\)90203-4](https://doi.org/10.1016/S0142-9612(98)90203-4).
12. Nishiguchi S, Kato H, Fujita H, Kim HM, Miyaji F, Kokubo T, et al. Enhancement of bone-bonding strengths of titanium alloy implants by alkali and heat treatments. *J Biomed Mater Res Appl Biomater.* 1999;48:689–96. doi: [10.1002/\(SICI\)1097-4636\(199948:5<689::AID-JBM13>3.0.CO;2-C](https://doi.org/10.1002/(SICI)1097-4636(199948:5<689::AID-JBM13>3.0.CO;2-C).
13. Nakagawa M, Zhang L, Udoh K, Matsuya S, Ishikawa K. Effect of hydrothermal treatment with CaCl<sub>2</sub> solution on surface property and cell response of titanium implants. *J Mater Sci: Mater Med.* 2005;16:985–91. doi: [10.1007/s10856-005-4753-0](https://doi.org/10.1007/s10856-005-4753-0).
14. Kim HM, Miyaji F, Kokubo T. Effect of heat treatment on apatite-forming ability of Ti metal induced by alkali treatment. *J Mater Sci: Mater Med.* 1997;8:341–7. doi: [10.1023/A:1018524731409](https://doi.org/10.1023/A:1018524731409).
15. Toumelin-chemla F, Rouelle F, Burdairon G. Corrosive properties of fluoride-containing odontologic gels against titanium. *J Dentistry.* 1996;24:109–15. doi: [10.1016/0300-5712\(95\)00033-X](https://doi.org/10.1016/0300-5712(95)00033-X).
16. Strietzel R, Hosch A, Kalbfleisch H, Buch D. In vitro corrosion of titanium. *Biomaterials.* 1998;19:1495–9. doi: [10.1016/S0142-9612\(98\)00065-9](https://doi.org/10.1016/S0142-9612(98)00065-9).
17. Nakagawa M, Matsuya S, Shiraiishi T, Ohta M. Effect of fluoride concentration and pH on corrosion behavior of titanium for dental use. *J Dent Res.* 1999;78:1568–72. doi: [10.1177/00220345990780091201](https://doi.org/10.1177/00220345990780091201).
18. Kaneko K, Yokoyama K, Moriyama K, Asaoka K, Sakai J, Nagimo M. Delayed fracture of beta titanium orthodontic wire in fluoride aqueous solutions. *Biomaterials.* 2003;24:2113–20. doi: [10.1016/S0142-9612\(02\)00642-7](https://doi.org/10.1016/S0142-9612(02)00642-7).
19. Schiff N, Grosogeat B, Lissac M, Dalard F. Influence of fluoride content and pH on the corrosion resistance of titanium and its



- alloys. *Biomaterials*. 2002;23:1995–2002. doi:[10.1016/S0142-9612\(01\)00328-3](https://doi.org/10.1016/S0142-9612(01)00328-3).
20. Huang HH. Effect of fluoride concentration and elastic tensile strain on the corrosion resistance of commercially pure titanium. *Biomaterials*. 2002;23:59–63. doi:[10.1016/S0142-9612\(01\)00079-5](https://doi.org/10.1016/S0142-9612(01)00079-5).
  21. Matono Y, Nakagawa M, Matsuya S, Ishikawa K, Terada Y. Corrosion behavior of pure titanium and titanium alloys in various concentrations of acidulated phosphate fluoride (APF) solutions. *Dent Mater J*. 2006;25:104–12.
  22. Nakagawa M, Matono Y, Matsuya S, Udoh K, Ishikawa K. The effect of Pt and Pd alloying additions on the corrosion behavior of titanium in fluoride-containing environment. *Biomaterials*. 2005;26:2239–46. doi:[10.1016/j.biomaterials.2004.07.022](https://doi.org/10.1016/j.biomaterials.2004.07.022).
  23. Nakagawa M, Matsuya S, Udoh K. Corrosion behavior of pure titanium and titanium alloys in fluoride containing solutions. *Dent Mater J*. 2001;20:305–14.
  24. Nakagawa M, Matsuya S, Udoh K. Effect of fluoride and dissolved oxygen concentrations on the corrosion behavior of pure titanium and titanium alloys. *Dent Mater J*. 2002;21:83–92.
  25. Yamazoe J, Nakagawa M, Matono Y, Takeuchi A, Ishikawa K. The development of Ti alloys for dental implant with high corrosion resistance and mechanical strength. *J Dent Mat*. 2007;26:260–7. doi:[10.4012/dmj.26.260](https://doi.org/10.4012/dmj.26.260).
  26. Ayukawa Y, Takeshita F, Inoue T, Yoshinari M, Ohtsuka Y, Murai K, et al. An ultrastructural study of the bone-titanium interface using pure titanium-coated plastic and titanium rod implants. *Acta Histochemica et Cytochemica*. 1996;29:234–54.
  27. Ayukawa Y, Okamura A, Koyano K. Simvastatin promotes osteogenesis around titanium implants. *Clin Oral Implant Res*. 2004;15:346–50. doi:[10.1046/j.1600-0501.2003.01015.x](https://doi.org/10.1046/j.1600-0501.2003.01015.x).
  28. Mano T, Ueyama Y, Ishikawa K, Matsumura T, Suzuki K. Initial tissue response to a titanium implant coated with apatite at room temperature using a blast coating method. *Biomaterials*. 2002;23:1931–6. doi:[10.1016/S0142-9612\(01\)00319-2](https://doi.org/10.1016/S0142-9612(01)00319-2).
  29. Takadama H, Kim HM, Kokubo T, Nakamura T. An X-ray photoelectron spectroscopy study of the process of apatite formation on bioactive titanium metal. *J Biomed Mater Res*. 2001;55:185–93. doi:[10.1002/1097-4636\(200105\)55:2<185::AID-JBM1005>3.0.CO;2-P](https://doi.org/10.1002/1097-4636(200105)55:2<185::AID-JBM1005>3.0.CO;2-P).
  30. Kim HM, Himeno T, Kawashita M, Lee JH, Kokubo T, Nakamura T. Surface potential change in bioactive titanium metal during the process of apatite formation in simulated body fluid. *J Biomed Mater Res*. 2003;67:1305–9. doi:[10.1002/jbm.a.20039](https://doi.org/10.1002/jbm.a.20039).
  31. Gottlander M, Albrektsson T. Histomorphometric studies of hydroxyapatite-coated and uncoated CP titanium threaded implants in bone. *Int J Oral Maxillofac Implants*. 1991;6:399–404.
  32. Matsui Y, Ohno K, Michi KI, Yamagata K. Experimental study of high-viscosity flame-sprayed hydroxyapatite coated and non-coated titanium implants. *Int J Oral Maxillofac Implants*. 1994;9:397–404.
  33. Nishiguchi S, Kato H, Fujita H, Oka M, Kim HM, Kokubo T, et al. Titanium metals form direct bonding to bone after alkali and heat treatments. *Biomaterials*. 2001;22:2525–33. doi:[10.1016/S0142-9612\(00\)00443-9](https://doi.org/10.1016/S0142-9612(00)00443-9).